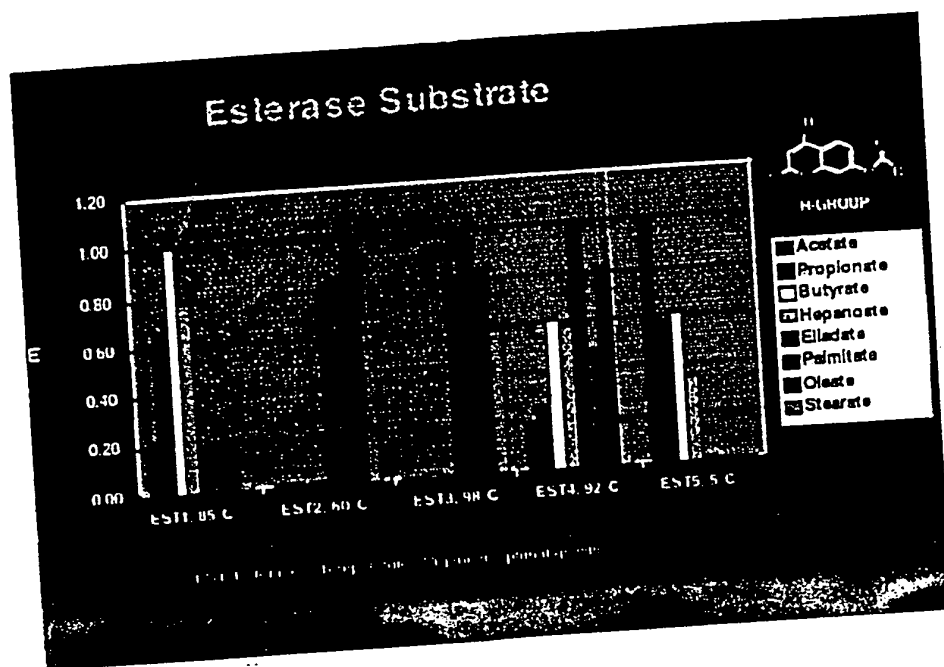
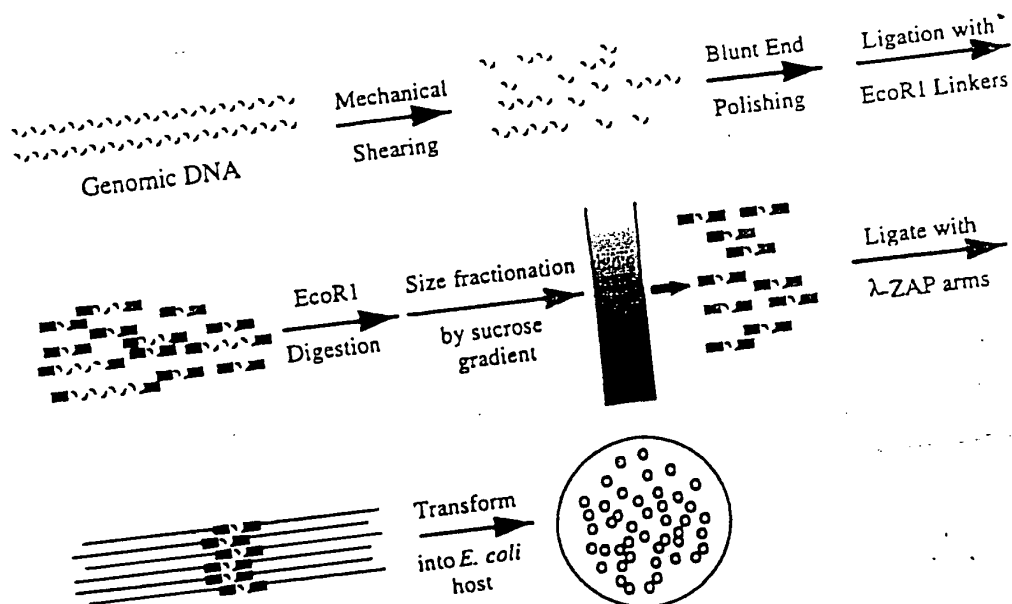


Figure 1



0063678-084100

Figure 2.



00636778 001100

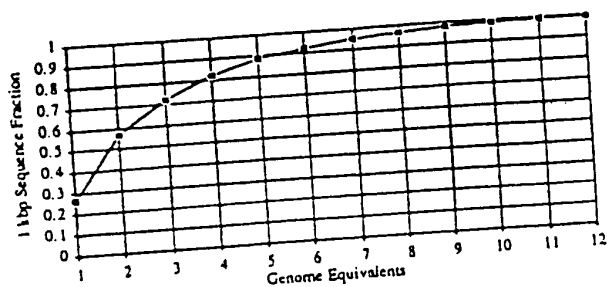


Figure 3.

001180-8449550

00120 8229550

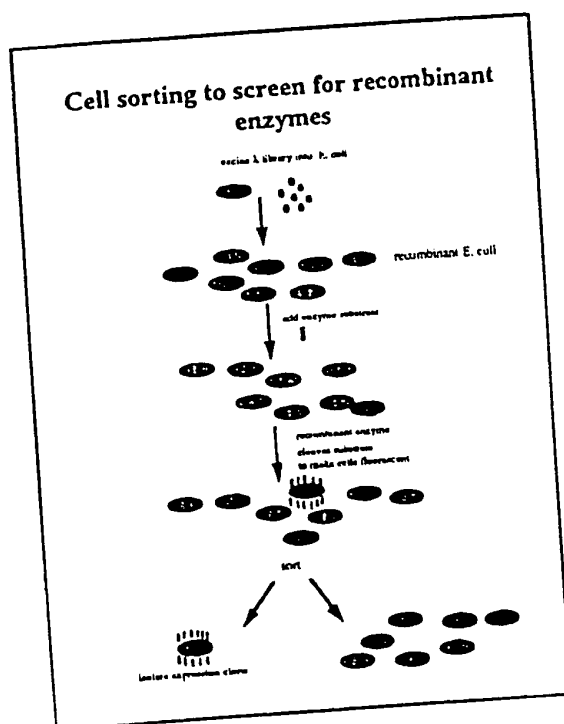
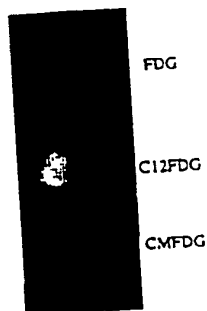


Figure 4.

**$\beta$ -Gal clone with different substrates**

- cells were stained with FDG, CMFDG or C12FDG, incubated for 30 min. at 70°C, spotted onto a slide and exposed to UV light.
- bright spot indicates staining of cells



E. coli expressing  $\beta$ -Gal from Sulfolobus spec. was grown over night. Cells were centrifuged and substrate was loaded with deionised water. After 5 min. cells were centrifuged and transferred into HEPES buffer and heated to 70°C for 30 min.. Cells were spotted onto a slide and exposed to UV light.

Figure 5

001180-82/95960

Figure 6

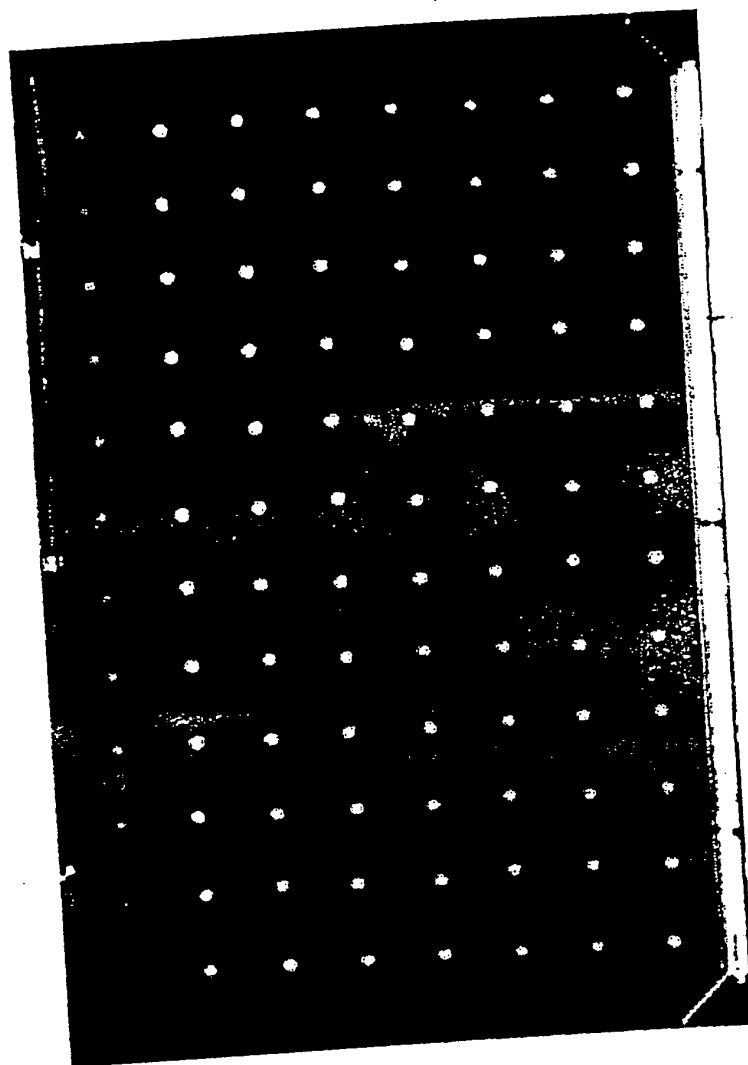
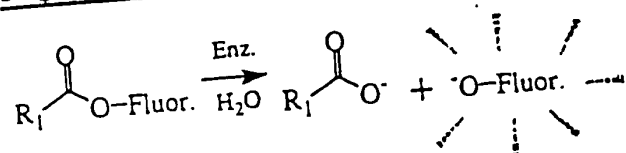


Figure 7



Principle type of fluorescence enzyme assay of deacylation.

00536778-004100

Figure 8

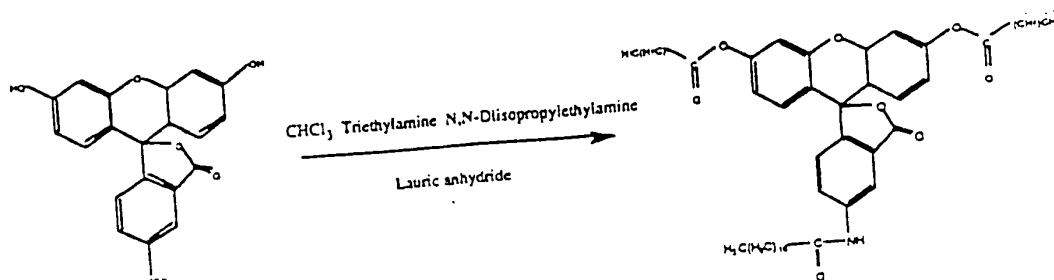


Staining of  $\beta$ -galactosidase clones from the hyperthermophilic archaeon *Sulfolobus solfataricus* expressed in *E.coli* using  $C_{12}$ -FDG as enzyme substrate.

00535778-001100



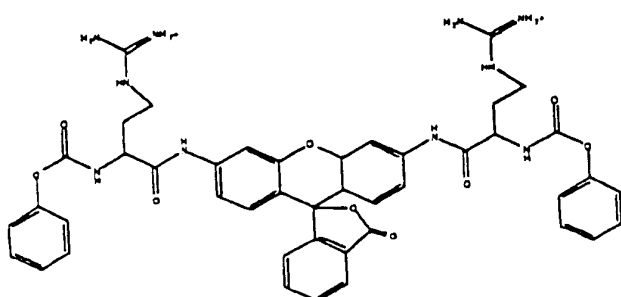
**Figure 9**



Synthesis of 5-dodecanoyl-aminofluorescein-di-dodecanoic acid

0053679 104100

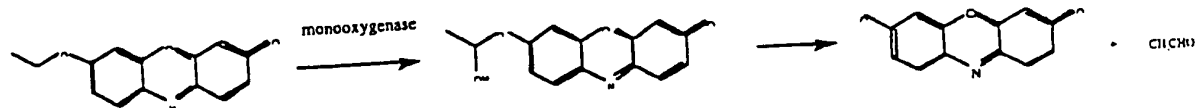
Figure 10



Rhodamine protease substrate.

00180-87495960

Figure 11



Compound and process that can be used in the detection of monooxygenases

00536778-081100

# Combinatorial Enzyme Development

(Natural + Non-natural Evolution)

Desired Enzyme

Improve Nature

Search Nature

Directed Evolution

Select Enzyme

New Enzyme

Enzyme Library

- Enzyme 1
- Enzyme 2
- Enzyme 3
- Enzyme 4
- Enzyme 5
- Enzyme 6
- Enzyme 7
- Enzyme 8
- Enzyme 9
- Enzyme 10
- Enzyme n

ID via High Throughput Screening

ID via Enzyme Characterization

ID via Mutation / Selection

NA Library

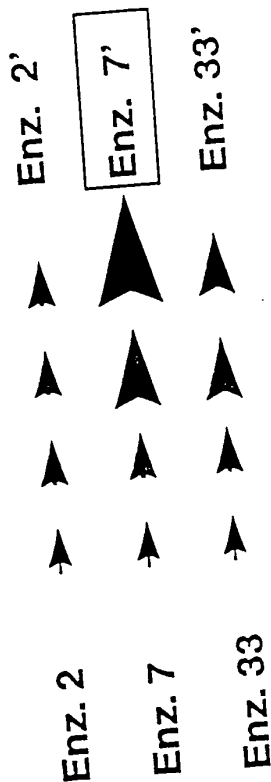


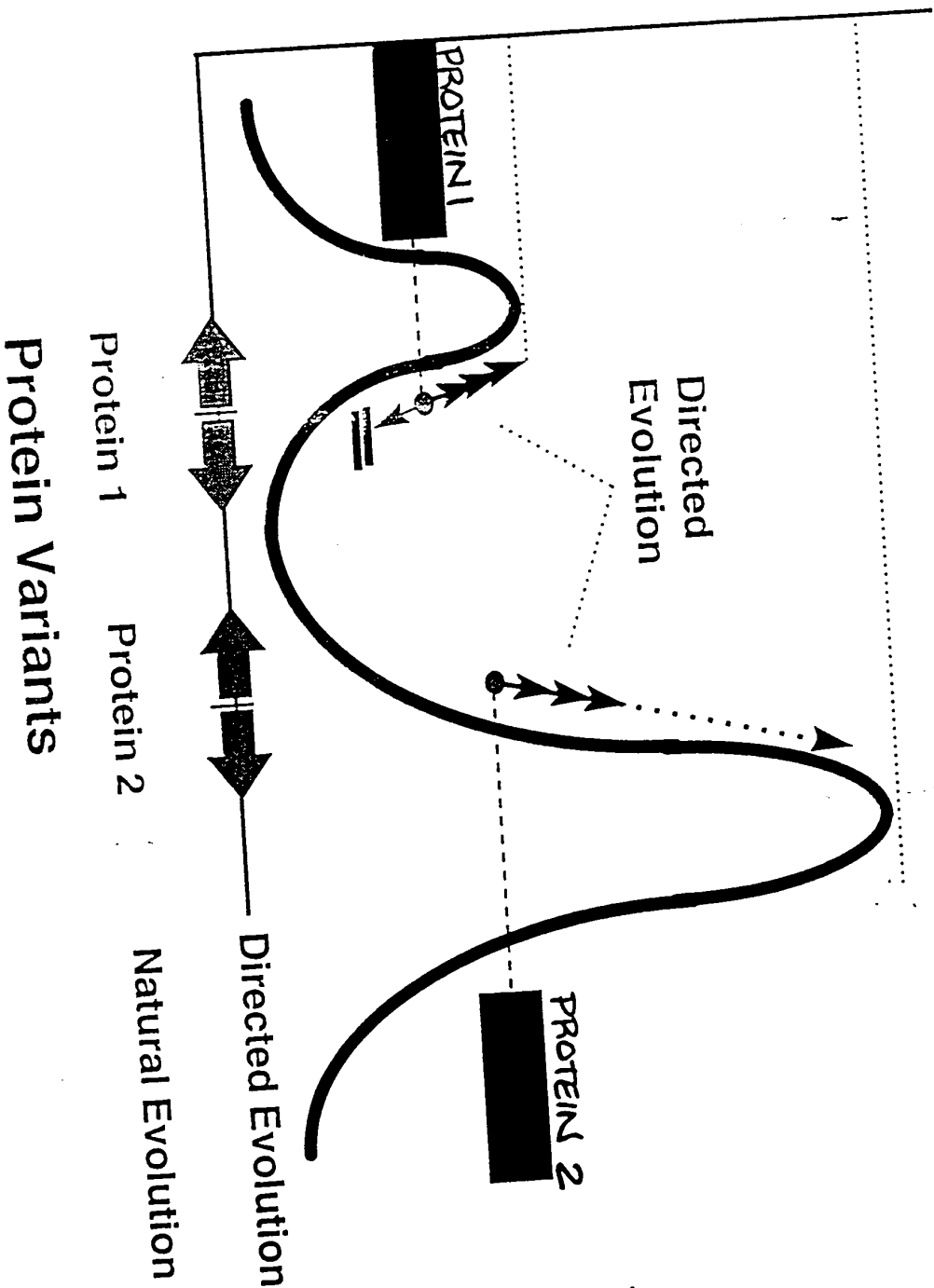
FIGURE 13

# Bypassing Barriers to Directed Protein Evolution

(Barrier = Capacity limit of directed evolution system)

- T STABILITY
- Solvent Stability
- Expression Level
- Buffer Compatibility
- Process Compatibility

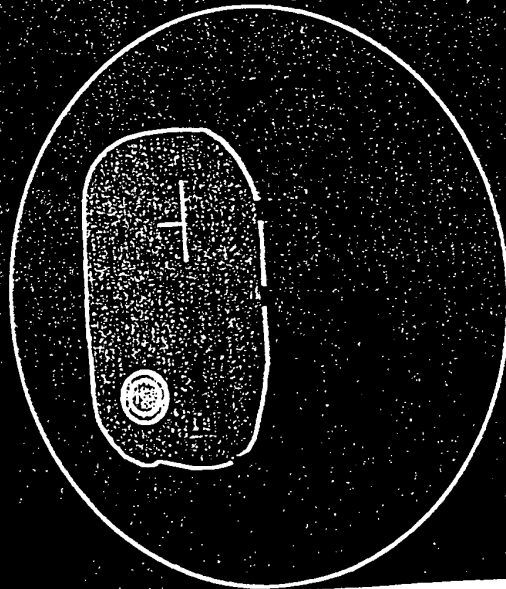
RELATIVE  
ENZYME  
ACTIVITY



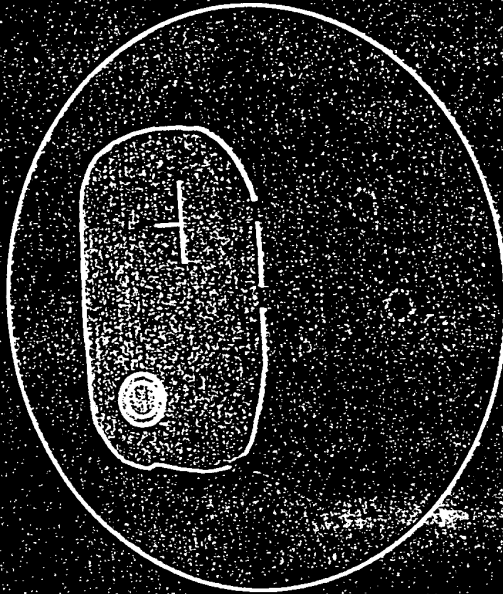
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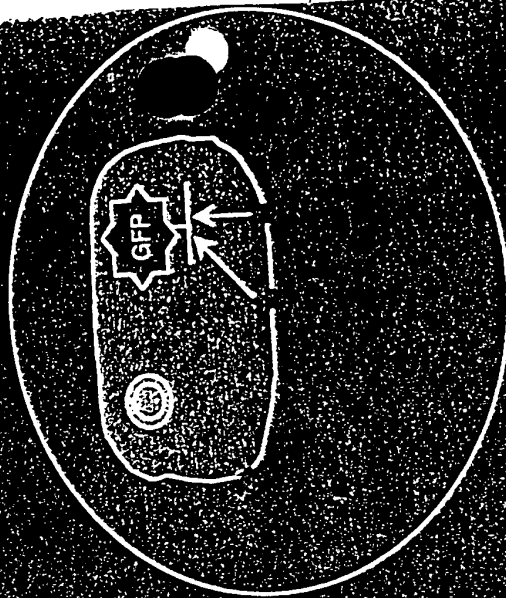
# Co-Encapsulation Assay for Novel Bioactive Discovery



Co-encapsulation  
Library + Eukaryote



Growth and expression  
of small molecule from host



Receptor binding of small  
molecule & GFP reporter

SM = Small molecule

L = Large insert library

E = Eukaryotic assay organism

R = Eukaryotic receptor

GFP = Green Fluorescent Protein

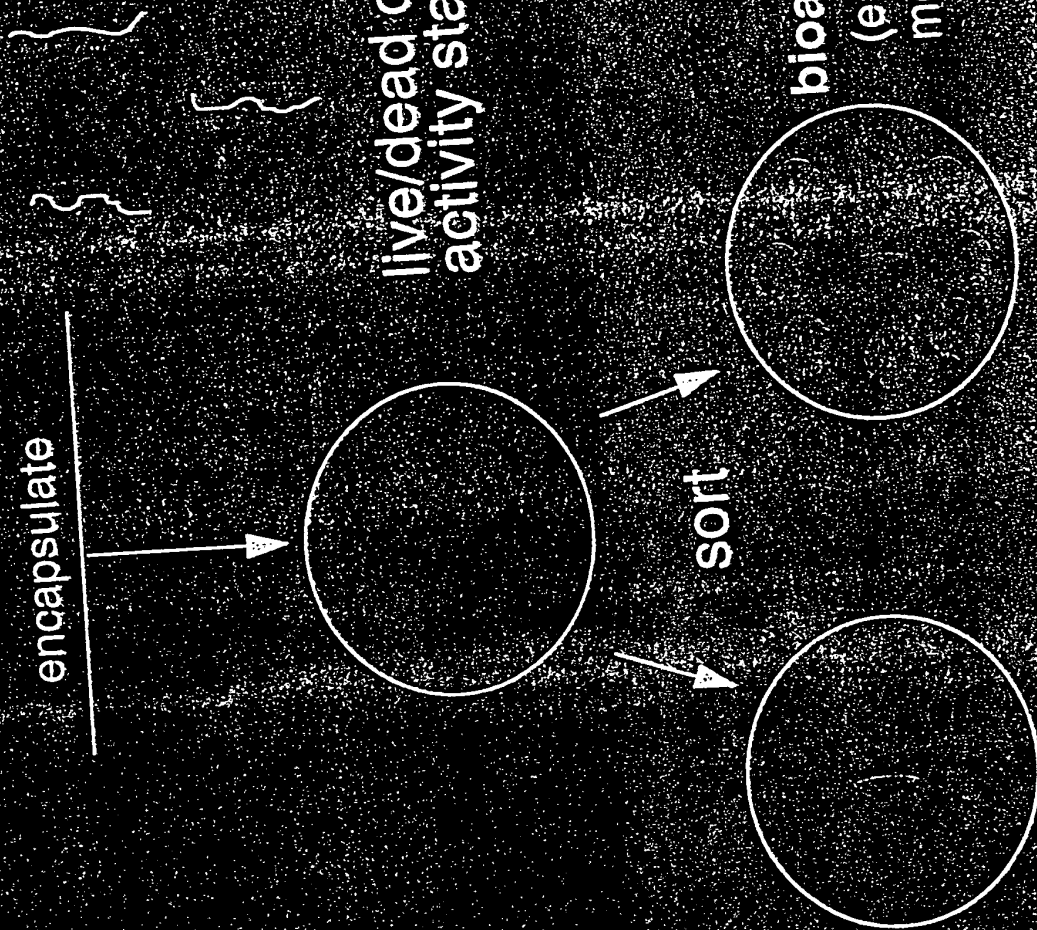


UNIVERSITY OF CAMBRIDGE

# FACS Screening for Encapsulation

## Test organisms

## Pathway clones









# FACS Enrichment for Gel Microdroplets Containing *S. diversa*

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